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Comprehensive GC-MS Profiling and Evaluation of Antimicrobial Properties in *Peperomia tetraphylla* (G. Forst.) Hook. & Arno

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ABSTRACT

The present study aimed at analyzing the phytochemical components with a GC-MS approach and to figure out the antimicrobial effectiveness of plant extracts against pathogenic bacterial strains (*Salmonella enterica*, *Pseudomonas sps*, *Streptococcus mutans*, *Staphylococcus aureus*) and pathogenic fungi (*Candida albicans*, *Aspergillus flavus*, *Aspergillus niger* and *Rhizopus oryzae*). A qualitative phytochemical evaluation of the entire plant indicated the presence of alkaloids, phenols, tannins, flavonoids, terpenoids, glycosides and so on. The qualitative phytochemical data were further validated by the GC-MS analysis of the entire plant crude extract. Thirty-two compounds, most significantly 1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z, β-Asarone, Phthalic acid, di(2-propylpentyl) ester and Bis (2-ethylhexyl) isophthalate were determined from the methanolic extract in the GC-MS evaluation. These chemicals had a variety of medicinal and antimicrobial properties. Furthermore, more research on *P. tetraphylla* is required due to its broad spectrum of beneficial pharmacological properties as a potential antimicrobial agent.

Key words: Phytochemical components, GC-MS approach, antimicrobial effectiveness, pharmacological activities

INTRODUCTION

Plants have long been employed as therapeutic agents, and there is a growing recognition of the importance of these medicinal floras. Secondary metabolites with intriguing biological functions are abundant in plants. Antibiotics have traditionally been derived from natural compounds derived from microbial sources. The importance of checking medicinal plants for active compounds has grown, nevertheless, as herbal medicine becomes more widely accepted as an alternative form of health care. Many plant species have considerable potential for therapeutic compounds that are currently undiscovered in the plant world. Individual, additive, or synergistic activities of phytochemicals to promote health make them effective in the treatment of specific illnesses. The discovery of bioactive ingredients drawn from natural reservoirs marks the start of innovative medication development. The systematic examination of botanical extracts is a ground-breaking strategy for identifying pharmacologically active compounds across a wide range of plant taxa. Flavonoids, tannins, saponins, alkaloids and terpenoids have a wide range of physiological effects, including but not limited to antioxidative, anti-inflammatory, anti-diarrheal, anti-ulcerative and anticancer properties. The approach of Gas Chromatography-Mass Spectrometry (GC-MS) is gaining interest for the evaluation of secondary metabolites found in medicinal plants. Because of its unparalleled applicability in the examination of essential oils, alcohols, acids, esters, alkaloids, steroids, amino compounds and nitro compounds, this technique has risen to prominence.

The Piperaceae family, which includes over 3700 species, is widespread in tropical and subtropical climates. In total, more than 1500 *Peperomia* species have been documented across the world, with *Peperomia tetraphylla* (G. Forst.) Hook. & Arn. have significant therapeutic importance. This plant has been used to cure a variety of ailments in

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traditional Chinese medicine, including cough, asthma, dysentery and diarrhea. The phytochemical profile of *P. tetraphylla* highlighted the presence of alkaloids, flavonoids, phenolic compounds, terpenoids, saponins, tannins and essential oil rich in terpenes and aromatic chemicals (Wang *et al.*, 2021). As a result, the current work seeked to assess the antimicrobial capacity, phytochemical screening, and gas chromatography-mass spectrometry (GC-MS) screening of several *P. tetraphylla* solvent extracts.

MATERIALS AND METHODS

The plant, *P. tetraphylla*, was gathered in August 2022 from Kommula Mamidi Village (18° 0' 55.548"N, 82° 29' 43.296"E, Elevation: 956 m), G. Madugula Mandal, Alluri Sitarama Raju district, Andhra Pradesh, India. A Taxonomist from the Department of Botany, Andhra University, validated the plant's identity and certified using voucher No. 25531. Whole plants were collected, shade-dried and powdered. From the ground plant material phytochemicals were extracted with different solvents (methanol, acetone, water, chloroform, and n-hexane) by using the Soxhlet extraction technique. Meanwhile, the extracts were collected in a rotary evaporator at 45°C under decreased pressure as a thick concentration. Each solvent extract underwent preliminary phytochemical analysis following the standard protocols.

The methanolic whole plant extract of *P. tetraphylla* was undertaken through Gas Chromatography-Mass Spectrometry (GC-MS) at the Sophisticated Analytical Instrument Facility (SAIF) laboratories, IIT Madras, utilizing a standard GCMS model as delineated hereafter. GC–MS system comprising an Agilent 8890 Gas Chromatograph coupled with an Agilent 5977 Mass Selective Detector (MSD). The transportation of samples was facilitated by helium gas maintained at a flow rate of 1.2 ml/min. The injection volume of 1 µl occurred at an elevated temperature of 280°C. The separation column, HP5, was characterized by dimensions of 30 m x 250 µm x 0.25 µm and exhibited a temperature gradient ranging from 75 to 360°C. The comprehensive run time for the gas chromatography procedure was established at 53.5 min. The ascertained phytochemical entities were characterized by comparing their mass spectrometry spectral patterns against the standard spectra cataloged in the NIST Mass Spectra Database, specifically employing the licensed NIST 2017 Library, and analyzed using Open Lab CDS 2.5 software.

In the case of bacteria, the agar well diffusion technique was employed to examine the antibacterial abilities of plant extracts on nutrient broth (NB) agar media. After inoculating the 100 µl bacterial culture into the prepared media and allowing it to solidify in the Petri plates, a sterilized L-shaped bent rod was used to equally spread it. With the use of a sterile cork borer, 5 mm diameter wells were created on the plates and filled with different solvent extracts (three concentrations) with positive and negative controls (20 µl each). After 24 h of incubation at 37°C, the zones of inhibition (mm) of the different extracts were measured, and the findings of each test were averaged after being performed three times. Throughout the microbiological experiment, a strict aseptic atmosphere was maintained. Fungal strains were grown on Potato Dextrose Agar (PDA) medium. The wells were made in the same way as before and filled with 20 µl of the sample solutions. The diameter of the inhibitory zone was measured after 48 h incubation period at 26°C. Fluconazole 30 µg/ml was used as a positive control for fungal cultures and streptomycin 100 µg/ml was used as a positive control for bacterial cultures. DMSO was used as a negative control for both bacterial and fungal cultures.

RESULTS AND DISCUSSION

The outcomes of the qualitative screening performed on the various *P. tetraphylla* extracts determined that they were comprised of various primary metabolites (carbohydrates, proteins as well as gums and mucilage) and secondary metabolites (alkaloids, phenols, tannins, flavanoids, terpenoids, glycosides, cardiac glycosides, saponins, steroids, anthocyanins and coumarins) as shown in Table 1. Methanol extracts exhibited the highest concentration of phyto compounds, followed by acetone and aqueous extracts, while chloroform extract contained a moderate amount, and n-hexane extract had the fewest active principles among the solvent extracts. Each of the five solvents

Table 1. Preliminary qualitative phytochemical assay of various extracts of *P. tetraphylla*

Plant constituents	Solvent extracts				
	Methanol	Acetone	Chloroform	Aqueous	n- hexane
Primary metabolites					
Carbohydrates	++	-	-	++	-
Proteins	+	-	-	+	-
Fixed oils and fats	-	-	-	-	-
Gums and mucilage	+	-	-	-	-
Secondary metabolites					
Alkaloids	+	-	+	-	-
Anthroqinones	-	-	-	-	-
Phenols	+	+	+	++	++
Tannins	++	++	+	-	+
Flavanoids	+	+	+	+	-
Terpenoids	-	+	-	+	+
Glycosides	-	+	-	-	+
Cardiac glycosides	+	-	+	+	-
Saponins	++	+	+	-	-
Steroids	+	+	+	-	+
Anthocyanins	+	+	-	+	-
Coumarins		-	-	+	-

included phenols and flavonoids. The acetone, chloroform and n-hexane extracts appeared to be devoid of primary metabolites. The previous study revealed that chloroform extracts of *P. tetraphylla* exhibited the highest presence of 9 distinct compounds, including alkaloids, carbohydrate, glycosides, flavonoids, phytosterols, fixed oils and fats, phenolic compounds, tannins and lignins. Acetone and alcoholic extracts contained eight common phytocompounds, while the aqueous extract had six compounds, and petroleum ether had the least four phytocompounds, including carbohydrates, phytosterols, fixed oils and fats, and lignins. In the current investigation 14 types of compounds were identified from five distinct solvent extracts of *P. tetraphylla* which could potentially be examined further for medicinal aspects.

The antimicrobial bioassay outcome, in opposition to pathogenic microorganisms, had been delineated based on the extent of the inhibition zone width utilizing crude extracts from the *P. tetraphylla* plant at concentrations of 10, 5 and 2.5 mg for each extract. The antibacterial observations have been tabulated and are presented in Table 2. The water exhibited the highest antibacterial activity, followed by the N-hexane extract. Despite methanol solvent had shown exceptional antibacterial action, acetone and chloroform extract demonstrated only modest activity. Except for methanol, none of the solvent extracts had antibacterial action against *S. typhi*. Except for *Pseudomonas*, N-hexane extract had no anti-bacterial action against *S. typhi*, *S. mutans* and *S. aureus*, whereas water extract had no activity against *S. typhi* and *S. mutans* and acetone extract had no activity just against *S. typhi*. Even though *S. typhi* was more resistant, *Pseudomonas* was the most sensitive to *P. tetraphylla* of the four pathogens studied.

It had been conclusively ascertained that ethanolic extracts originating from *Peperomia pellucida* manifest noteworthy antibacterial effects against *Escherichia coli* and *Staphylococcus aureus*. These effects were

Table 2. Antibacterial activity of different extracts of *P. tetraphylla*

Extract	*Salmonella typhi*			*Pseudomonas*			*Streptococcus mutans*			*Staphylococcus aureus*		
	10 mg	5 mg	2.5 mg	10 mg	5 mg	2.5 mg	10 mg	5 mg	2.5 mg	10 mg	5 mg	2.5 mg
Me	6.6±0.57	6.3±0.57	-	9±1	11.3±0.57	11.6±0.57	10.6±0.57	7±0	6.6±0.57	8.6±0.57	8.3±0.57	7.3±0.57
Ac	-	-	-	10.3±0.57	8±1	7.6±0.57	7±0	6.67±0.57	6.3±0.57	12±1	8.6±0.57	7.3±0.57
Ch	-	-	-	11.6±0.57	11±1	9.6±0.57	7±0	6.6±0.57	6.3±0.57	10±0	8.6±0.57	7.3±0.57
Aq	-	-	-	10.3±0.57	8.3±0.57	-	-	-	-	7.6±0.57	-	-
NH	-	-	-	7.6±0.57	7±0	-	-	-	-	-	-	-

Me: Methanol extract, Ac: Acetone extract, Ch: Chloroform extract, Aq: Aqueous extract and NH: N-Hexane extracts. Values represent mean ± standard deviations; "-" for no zone of inhibition. A zone of inhibition with a diameter of less than 6 mm was considered inactive.

quantified by a substantial mean inhibition zone diameter of 15.43±0.67 mm and 13.22±0.34 mm, respectively, as reported by Jessica *et al.* in 2020. In a separate study, the efficacy of an ethyl acetate leaf extract derived from *Peperomia borbonensis* was observed against both gram-negative bacteria (*Salmonella enterica*: 8.77±0.49, *Pseudomonas aeruginosa*: 8.77±0.40, *Escherichia coli*: 9.87±0.81) and gram-positive bacteria (*Listeria monocytogenes*: 9.20±1.11) at various extract doses (Dorla *et al.*, 2019). To the best of the available information, no investigation into the antibacterial activity against bacteria has been conducted on *P. tetraphylla* and this is the latest examination of the antibacterial activity of *P. tetraphylla* extracts.

The antifungal activity of various extracts of *P. tetraphylla* was assessed against *Candida albicans*, *Aspergillus flavus*, *Aspergillus niger* and *Rhizopus oryzae* at concentrations of 10, 5 and 2.5 mg. The methanol (Me) extract exhibited notable inhibitory effects against *C. albicans* (7.3±0.57 mm) and *A. niger* (10.6±0.57 mm) at 10 mg, while the acetone (Ac) extract demonstrated significant inhibition against *C. albicans* (8.6±0.57 mm) at 10 mg. The chloroform (Ch) extract displayed substantial antifungal activity against *C. albicans* (13±1 mm) and *R. oryzae* (8±1 mm) at 10 mg. The aqueous (Aq) extract showed noteworthy inhibitory effects against *C. albicans* (12±1 mm), *A. flavus* (7.6±0.57 mm), *A. niger* (10±1 mm) and *R. oryzae* (8.6±0.57 mm) at 10 mg. The n-hexane (NH) extract exhibited inhibitory effects against *C. albicans* (8.6±0.57 mm) at 10 mg (Table 3).

Earlier investigations indicated the efficacy of methanol leaf extract of *P. pellucida* against *C. albicans* and *R. stolonifera*, with fungal suppression demonstrated at doses ranging from 25 to 200 mg/ml. The n-hexane fraction, excluding *R. stolonifera* and *P. notatum*, inhibited all bacteria at concentrations ranging from 50 to 200 mg/ml, while the aqueous fraction exhibited minimal inhibition against *C. albicans* and *A. niger* at 50-200 mg/ml. Additionally, a separate study revealed the effectiveness of ethyl acetate leaf extract from *P. borbonensis* against the fungal strain *Aspergillus fumigates*, attaining a region of inhibition of approximately 7.97±0.67, thus confirming its antifungal properties (Dorla *et al.*, 2019). Copaene, Spathulenol, Isoaromadendrene epoxide, β-Asarone, and Phthalic acid, di (2-propylpentyl) ester, identified through gas chromatography-mass spectrometry analysis of the methanolic extract from the current study, have been previously reported with antimicrobial activities. The presence of these compounds may contribute to the notable antimicrobial action observed in this plant. This present investigation was undertaken to substantiate the antifungal capacity of *P. tetraphylla*, as no prior antifungal studies on this plant have been conducted to the best of knowledge.

Methanol extracts were selected for GC-MS evaluation to investigate the phytochemical composition since they demonstrated the greatest antimicrobial activity when compared with the other solvent extracts. This work showed that several physiologically active chemicals were found at varying concentrations in P. *tetraphylla* methanol extract and also provided an in-depth knowledge of the phytochemical profile that might be used to develop plant-based medicines. The methanol extracts' GC/MS spectrum data revealed multiple peaks (Fig. 1) indicating the presence of 28 distinct chemicals with retention times ranging from 7.457 to 41.407. The mass fragmentation patterns and retention indices from the Spectral Library and Database (NIST

Table 3. Antifungal activity of different extracts of *P. tetraphylla*

Extract	*Candida albicans*			*Aspergillus flavus*			*Aspergillus niger*			*Rzihopus oryzae*		
	10 mg	5 mg	2.5 mg	10 mg	5 mg	2.5 mg	10 mg	5 mg	2.5 mg	10 mg	5 mg	2.5 mg
Me	7.3±0.57	7.3±0.57	6±0	-	-	-	10.6±0.57	8.6±0.57	7.6±0.57	7.6±0.57	-	-
Ac	8.6±0.57	8.3±0.57	7±0	-	-	-	-	-	-	-	-	-
Ch	13±1	8±0	7.6±0.57	-	-	-	-	-	-	8±1	7.6±0.57	-
Aq	12±1	8.6±0.57	7±1	7.6±0.57	6.6±0.57	6.3±0.57	10±1	7.6±0.57	6.6±0.57	8.6±0.57	7.6±0.57	6±0
NH	8.6±0.57	8.3±0.57	7.3±0.57	-	-	-	-	-	-	-	-	-

Me: Methanol extract, Ac: Acetone extract, Ch: Chloroform extract, Aq: Aqueous extract and NH: N- Hexane extracts. Values represent mean ± standard deviations; "-" for no zone of inhibition. A zone of inhibition with a diameter of less than 6 mm was considered inactive.

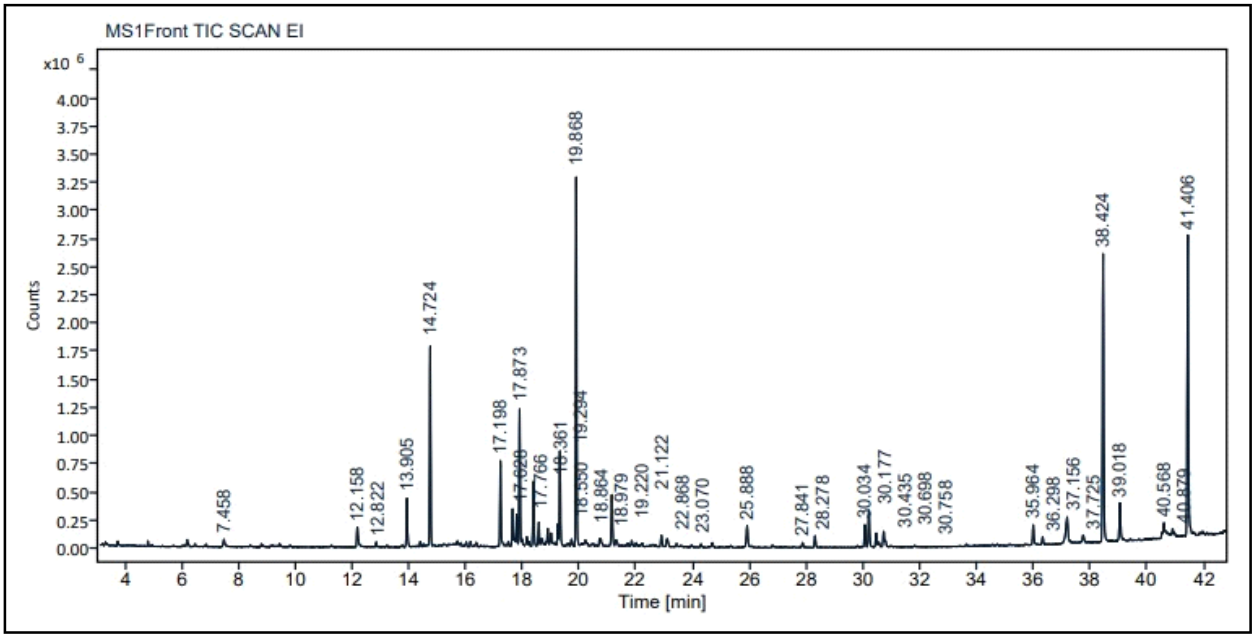

Fig. 1. GC-MS chromatogram of *P. tetraphylla*'s methanol extract.

2017 Library) were used to identify and describe each of these compounds. Table 4 lists the active components in chronological sequence of retention lengths, as well as their peak area percentage, molecular formula and molecular weight. A review of the literature was done to investigate the biological impacts of significant chemicals.

Following the reported biological properties, the compounds discovered in this study were mostly said to have anti-tumor, antibacterial, anti-inflammatory, analgesic, antineoplastic, anti-virulence, antioxidant effects, antifungal, for the treatment of menstrual disorders, abortifacient, antiandrogenic and anticancer effects. The phyto compound 1,3-benzene dicarboxylic acid, bis (2-ethylhexyl) ester was most prevalent in the *P. tetraphylla* extract, with an area percentage of 16.24% and a retention time of 41.407, and it had biological functions such as anti-cancer efficacy followed by Asarone peak value of 15.93% with retention time 19.86 analgesic and neuroprotective properties and the third significant peak was attained by Phthalic acid, di(2-propylpentyl) ester with area percentage of 14.73% with retention time 38.42 which had anti-microbial activity (Nabi *et al.*, 2022). The fourth one was 1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z with an area percentage of 8.74% and a retention time of 14.72 which had anti-microbial activity.

Three compounds, specifically copaene, caryophyllene and asarone, identified in *P. obtusifolia*, were detected in *P. tetraphylla*, suggesting potential biochemical similarities between the two species. The gas chromatography-mass spectrometry (GC-MS) analysis of the methanol extract of *P. pellucida* revealed 16 compounds, including caryophyllene, hexadecanoic acid methyl ester, and phthalic acid, di(2-propylpentyl) ester, which were also found in *P. tetraphylla* (Rajesh Babu *et al.*, 2023). Additionally, another investigation identified 17 chemical components in *P. tetraphylla* alcohol extracts, encompassing four secolignans, seven flavonoids, two alkaloids, one tetrahydrofuranlignan and three phenolic acids (Wang *et al.*, 2021). To the best of knowledge, no GC-MS screening was conducted on *P. tetraphylla*. In contrast to previous studies, this research presented the latest analysis of the GC-MS profile of the methanolic extracts of *P. tetraphylla.*

CONCLUSION

The phytochemicals in *P. tetraphylla* whole plant extract were qualitatively analyzed in methanol, acetone, chloroform, aqueous and n-hexane, which demonstrated to contain numerous primary metabolites and secondary metabolites bearing diverse pharmacological activities. The GC-MS screening of *P.*

Table 4. Bioactive chemical profile of *P. tetraphylla* through GC-MS analysis

Compound name	Rt minutes	Mol. weight	Mol. formula	Area (%)	Importance
Phenol, 2,6-dimethoxy	12.158	154.16	$C_8H_{10}O_3$	1.3	Anti-oxidant
Copaene	12.820	204.35	$C_{15}H_{24}$	0.2	Antimicrobial and antioxidant
Caryophyllene (β- caryophyllene)	13.902	204.26	$C_{15}H_{24}$	2.4	Anti-inflammatory activity, pain relieving, and anti-oxidant
1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z	14.727	204.35	$C_{15}H_{24}$	8.74	Anti-aging, anti-microbial
1,2-Dimethoxy-4-(2-methoxyethenyl)benzene	17.196	194.23	$C_{11}H_{14}O_3$	3.98	
Spathulenol	17.627	220.35	$C_{15}H_{24}O$	1.41	Anti-bacterial, anti-oxidant and anti-inflammatory (Nascimento *et al.*, 2018)
Isoaromadendrene epoxide	17.765	220.35	$C_{15}H_{24}O$	1.02	Anti-microbial, anti-oxidant and anti-inflammatory
β-Asarone	17.871, 18.553, 19.296	208.25	$C_{12}H_{16}O_3$	5.75	Anti-fungal (Ramya *et al.*, 2017)
Humulene epoxide	18.359	220.35	$C_{15}H_{24}O$	3.18	
Humulenol-II	18.865	220.35	$C_{15}H_{24}O$	0.71	
Isospathulenol	18.978	220.35	$C_{15}H_{24}O$	0.59	
Benzene, 1,2,3-trimethoxy-5-(1-propenyl)-, (E)-	19.221	208.25	$C_{12}H_{16}O_3$	0.51	
Asarone	19.865	208.25	$C_{12}H_{16}O_3$	15.9	Analgesic, Neuroprotetive
Alloaromadendrane-4beta,10alpha-diol	21.122	238.37	$C_{15}H_{26}O_2$	2.60	
Benzamide, 3,4,5-trimethoxy	22.866	211.21	$C_{10}H_{13}NO_4$	0.50	
Hexadecanoic acid, methyl ester	25.885	270.45	$C_{17}H_{34}O_2$	1.39	Anti-bacterial
1,3-Dioxolo[4,5-g]isoquinolin-5-ol, 5,6,7,8-tetrahydro-4-methoxy-6-methyl	27.842	222.26	$C_{12}H_{16}NO_3$	0.26	
2-Propenoic acid, 3-(3,4,5-trimethoxyphenyl)-, methyl ester	28.279	256.3	$C_{13}H_{16}O_5$	0.60	
Methyl 9-cis,11-trans-octadecadienoate	30.036	279.4	$C_{18}H_{31}O_2$	1.13	Anti-cancer and anti-oxidant (Shoge and Amusan, 2020)
11-Octadecenoic acid, methyl ester	30.180	296.5	$C_{19}H_{36}O_2$	1.86	Anti-microbial and Antidiarrheal (Shoge and Amusan, 2020)
Phytol	30.436	296.5	$C_{20}H_{40}O$	0.64	Antioxidant, anti-inflammatory, and antiallergic (Carvalho *et al.*, 2020)
Heptadecanoic acid, 16-methyl-, methyl ester	30.755	298.5	$C_{19}H_{38}O_2$	0.23	
Hexanedioic acid, dioctyl ester	35.962	370.6	$C_{22}H_{42}O_4$	1.06	
Pregna-4,9(11)-dien-20-ol-3-on-19-oic acid lactone	36.299	326.4	$C_{21}H_{26}O_3$	0.40	
Pregna-4,9(11)-dien-20-ol-3-on-19-oic acid lactone	37.156	326.4	$C_{21}H_{26}O_3$	2.58	
Phthalic acid, di(2-propylpentyl) ester	38.425	390.6	$C_{24}H_{38}O_4$	14.7	Anti-microbial (Osuntokun and Cristina, 2019)
Ursolic aldehyde	39.019	440.7	$C_{30}H_{48}O_2$	2.20	
9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester	40.569	356.5	$C_{21}H_{40}O_4$	0.94	
1,3-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester	41.407	390.5	$C_{24}H_{38}O_4$	16.2	Anti-cancer activity

tetraphylla indicated the existence of 32 bioactive compounds with a wide variety of pharmacological prospects, including antibacterial, analgesic, neuroprotective, anticancer and so on. These bioactive chemicals in *P. tetraphylla* contribute to a variety of therapeutic and pharmacologic effects found in conventional medicine. Additional investigation is needed to isolate a specific component that ends up in a favourable result in a biological assay, and adequate procedures for in-depth investigations should be established. In conclusion, *P. tetraphylla* stands out as a repository of diverse phytochemical constituents, encompassing alkaloids, flavonoids, phenolic compounds, terpenoids, essential oils, saponins and tannins. These bioactive compounds offer a foundation for potential therapeutic applications, warranting further investigation to unlock their complete pharmacological potential.

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